Normal Postpartum Involution of the Uterus in the Dog

M.A. Al-Bassam, R.G. Thomson and L. O'Donnell*

ABSTRACT

Ninety-eight reproductive tracts from dogs at different postpartum time periods were used to investigate stages of normal involution. Seventy-eight reproductive tracts were obtained from the field, and 20 obtained surgically for gross and microscopic examination. Plasma progesterone was measured in 22 dogs at various times postpartum.

The uterine horns during the first week postpartum were dilated and edematous. The placental sites were 1.5-3 cm in width, rough, granular and covered with mucus and a few blood clots. By the fourth week the placental sites were thick, grayish-tan and nodular with a few blood clots within nodules. The uterine horns during the seventh week were greatly contracted and the placental sites were narrow and light in color. A few nodules were still present on the surface. By the ninth week the uterine horns were uniform in shape and contracted with a narrow lumen. The placental sites appeared as a narrow brown band.

Histologically the placental sites during the first week postpartum were covered by an eosinophilic staining necrotic mass and a few intact epithelial cells scattered on the surface as an interrupted single layer. Under the necrotic mass, large eosinophilic staining cells in moderate number were scattered throughout the lamina propria of the placental site. These cells were considered to be decidual cells. By the fourth week the placental sites were covered by a large lobulated mass of collagen fibers. The uterine glands were greatly dilated and degenerate, and mononuclear cell infiltration in the lamina propria was pronounced. By the seventh week, large masses of collagen fibers were detached from the surface, and endometrial glands were normal in size and shape. By the ninth week surface sloughing was completed. However, regeneration and replacement of the endometrial lining from the mouth of the uterine glands continued until the end of the twelfth week when the involution process was completed.

The progesterone levels were very low for eight weeks postpartum.

RÉSUMÉ

Cette expérience consistait à étudier les aspects macroscopiques et microscopiques des stades de l'involution utérine canine normale, à différentes périodes ultérieures à la parturition. On utilisa à cette fin les organes génitaux internes de 98 chiennes; 78 de ces échantillons provenaient de cliniques vétérinaires et 20, de cas de chirurgie expérimentale du Collège Vétérinaire de Guelph. On mesura aussi la teneur en progestérone du plasma de 22 chiennes, à différentes périodes ultérieures à la parturition.

Au cours de la semaine qui suivit la parturition, les cornes utérines s'avérèrent dilatées et oedémateuses. Les sites placentaires mesuraient de 1,5 à 3 cm de largeur; ils étaient rugueux, granuleux et recouverts de mucus et de quelques caillots sanguins. Au cours de la quatrième semaine, les sites placentaires présentaient une couleur gris-brun et des nodules qui recelaient quelques caillots sanguins. Au cours de la septième semaine, les cornes utérines affichaient une contraction appréciable et les sites placentaires étaient étroits et pâles; leur surface présentait encore quelques nodules. Au cours de la neuvième semaine, les cornes utérines affichaient un retour à la normale, tandis que les sites placentaires ne correspondaient plus qu'à une bande brune et étroite.

L'examen microscopique des sites placentaires, au cours de la semaine ultérieure à la parturition, révéla qu'ils étaient recouverts par une masse nécrotique éosinophile et par quelques cellules épithéliales intactes, éparpillées superficiellement comme une couche simple, mais incomplète. Sous la masse nécrotique, le chorion des sites placentaires contenait un nombre modéré de cellules volumineuses et éosinophiles; ces cellules éparpillées semblaient correspondre à des cellules déciduales. Au cours de la quatrième semaine, les sites placentaires étaient recouverts d'une masse volumineuse et lobulée de fibres collagènes; les glandes utérines affichaient par ailleurs une dilatation marquée

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et de la dégénérescence; la chorion présentait une infiltration importante de mononucléaires. Au cours de la septième semaine, des masses importantes de fibres collagènes se détachaient de la surface des sites placentaires et les glandes endométriales retrouvaient progressivement leurs dimensions et leur forme normales. Au cours de la neuvième semaine, la desquamation de la surface des sites placentaires se compléta; la régénération et le remplacement de l'épithélium de la muqueuse utérine, à partir de l'embouchure des glandes utérines, continua cependant jusqu'à la fin de la 12e semaine qui marqua le complètement du processus d'involution utérine. La teneur du plasma en progestérone se révéla très basse, tout au long des huit semaines ultérieures à la parturition.

INTRODUCTION

In spite of the abundant literature regarding the canine placenta, only a few reports describe normal uterine postpartum involution in the bitch. Involution has been described briefly as a part of the estrous cycle in the dog (3, 13). Both reports indicate that twelve weeks were necessary for the completion of involution. During the first week postpartum, the placental sites were difficult to recognize grossly due to marked contraction of the myometrium (3), and by the fourth week postpartum the endometrium at the interplacental sites incurred extensive sloughing and desquamation, whereas the desquamation at the placental site started later, and was more pronounced.

This paper describes in some detail the gross and microscopic features during postpartum involution of the placental and interplacental areas of the uterus in the dog.

MATERIALS AND METHODS

In order to obtain as many samples as possible, reproductive tracts were obtained from two sources, local veterinarians and experimental surgical cases from the college. The intention was to secure as many samples as possible in order to have a broad coverage of the postpartum period.

Seventy-eight reproductive tracts from different breeds and ages of dogs with or without history of uterine diseases fixed in 10% formalin solution, were obtained from local practices. Fifty-three samples were from dogs up to three months postpartum, ten more than three months, 12 samples at different stages of pregnancy, and three had pyometra. The gross and microscopic examinations were mostly concerned with the samples from dogs up to three months postpartum (Table I).

Twenty pregnant or recently postpartum bitches of different ages and breeds were obtained and uterine samples were removed surgically at specific time periods. From four dogs, four to six uterine samples were taken surgically at different times postpartum. In the remainder, only two surgical procedures were performed; one uterine horn was removed first, and later the other horn and the two ovaries were removed. Forty-six uterine samples were obtained surgically at intervals over eight weeks postpartum.

GROSS EXAMINATION

The number of placental sites in each sample and the size and gross changes of each placental site were recorded. The number and size of corpora lutea from each dog were also recorded.

TABLE I. Postpartum Time Period Distribution in Specimens from Field and Surgical Cases

Week	Surgical Cases	Field Cases
1	9	3
2	6	—
3	7	2
4	7	2
5	4	3
6	4	1
7	6	6
8	2	8
9	—	10
10	—	4
11	—	3
12	—	11
Totals	45	53

MICROSCOPIC EXAMINATION

Three placental sites were selected from each uterine sample (except the first four surgical dogs), and transverse or longitudinal incisions were made through the sites and interplacental uterine tissues. One of the ovaries from each case was examined microscopically. Tissues were embedded in paraffin, sectioned at 5 μm and stained with hematoxylin and eosin.

Masson's trichrome staining method (15), was used to distinguish the presence of collagen from other components. Perl's staining method (16), was used to determine the presence of hemosiderin. In order to detect differences in the microscopic structure between different areas of the placental site, semiserial 5 μm sectioning from 15 selected blocks was carried out, and the sections stained with hematoxylin and eosin. From ten selected blocks, 5 μm serial sections extending from the myometrium to the endometrial lumen were stained with hematoxylin and eosin. This procedure was carried out to determine if there was closure or narrowing in the glandular ducts.

HORMONAL ASSAY

A single 10 mL blood sample was collected into evacuated tubes containing 143 USP units of sodium heparin solution at the time of the surgical procedures by cephalic venipuncture. Plasma was obtained by centrifugation and stored frozen until assayed. Plasma progesterone levels were determined in ten samples by using the competitive protein binding assay (18, 19), utilizing a rapid method of sample preparation (14). A radioimmunoassay method (5) was used in the remaining 12 samples for the determination of the plasma progesterone levels. Circumstances were such that the samples had to be processed in two different laboratories and consequently two different methods.

RESULTS

GROSS FINDINGS

During the first week postpartum, the uterus was still dilated, edematous, and signs of contraction were seen on the serosal surface as small longitudinal folds (Fig. 1). The mucosal surface at the interplacental site was folded and covered with dark brown mucus. The placental sites were 1.5-2 cm in width and their surface was rough, granular and covered with dark brown mucus and a few blood clots. The edges of the placental sites were long, folded inward and covered part of the placental site (Fig. 2).

During the second and third week the uterus was smaller. The placental sites were visible from the serosal surface as swellings in the uterine wall. The entire mucosal surface was still covered by a dark brown mucus but less in comparison to the first week. The placental sites were thicker and their surface was granular, grayish-tan and mixed with blood clots. The edges of the placental sites were smaller in size.

By the fourth week the uterus was much smaller and the placental sites appeared on the serosal surface as ellipsoid swellings (Fig. 3). The mucosal surface of the interplacental sites was less folded, and was covered with clear mucus. The placental sites were grayish-tan and contained eight to 12 small grayish nodules (0.2-0.4 cm diameter) spread randomly over the placental site (Fig. 4). A few pinpoint hemorrhagic foci and blood clots were seen within the nodules.

By the fifth week the uterine horn had become much smaller in diameter. The placental sites appeared as small ellipsoid swellings on the serosal surface, and on the mucosal surface the placental sites were smaller in size. During the sixth and seventh weeks the mucosal surface of the placental sites were light brown and narrow (0.8-1.5 cm in width). About six to eight grayish nodules were still present on the surface. No hemorrhagic foci or blood clots were present. By the eighth week, the diameter of the uterine horns reached its smallest size. The horns were uniform in shape with small ellipsoid shaped placental sites visible from the serosal surface (Fig. 5). On the mucosal surface, the number of grayish nodules were fewer (three or four) at the placental sites (Fig. 6). By the ninth week the uterine horns were uniform in shape with a narrow lumen. The placental sites were only differentiated from the

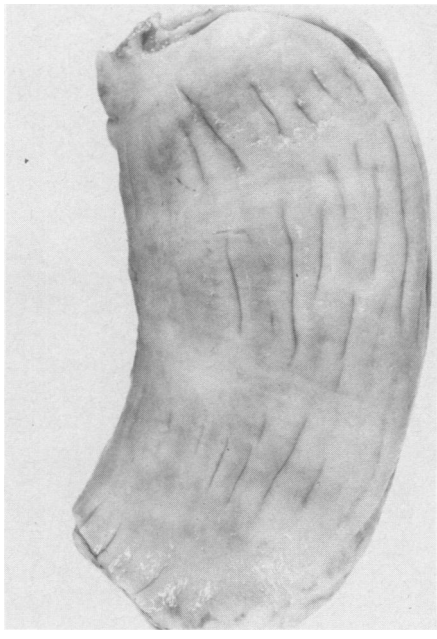

Fig. 1. Gross changes in a uterine horn one week postpartum. The horn is dilated, and signs of contraction appear on the serosal surface as small longitudinal folds.

Fig. 2. Endometrium one week postpartum. The mucosal surface between the placental sites is folded and covered with dark brown mucus. Placental site (arrows) are rough, 1-1.5 cm in width, and large mucosal folds cover part of them.

Fig. 3. Uterine horn, serosal surface four weeks postpartum. Placental site is visible clearly from serosal surface as an ellipsoidal swelling.

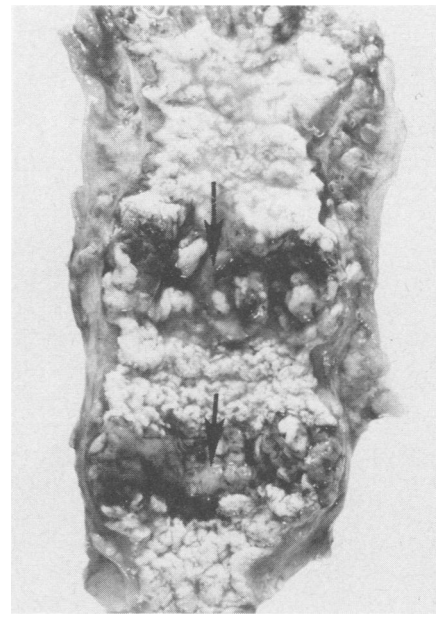

Fig. 4. Uterine horn, mucosal surface four weeks postpartum. The surface is less folded and covered with clear mucus. Placental sites (arrows) are grayish-tan. Eight to twelve small grayish nodules are spread over each site.

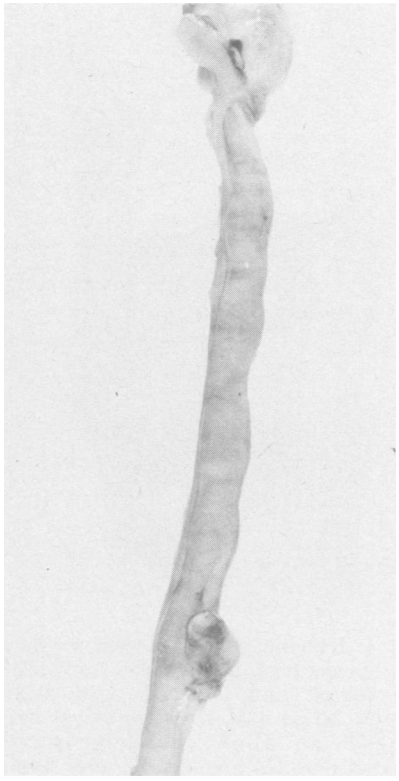

Fig. 5. Uterine horn, serosal surface eight weeks postpartum. The uterine horn is greatly contracted and narrower in diameter.

Fig. 6. Uterine horn mucosal surface eight weeks postpartum. Placental sites are small and three to four grayish nodules are present per site.

interplacental endometrium by the brown color.

MICROSCOPIC FINDINGS

Placental Site Endometrium — At parturition the plane of separation of the placenta usually lay in the distal portion of the spongy layer where there were only thin walled partitions between the greatly dilated glands (Fig. 7).

The histological structure of the placental site from parturition to seven days postpartum was as follows. After the separation of the placenta, the basal glandular zone was the only viable and intact layer remaining (Figs. 8 and 9). At the plane of placental separation over the basal glandular zone a necrotic mass was observed with a few intact, large and foamy epithelial cells scattered on the surface as an interrupted single layer (Figs. 8 and 9). These cells were considered to be remnants from the basal part of the separated spongy layer. The eosinophilic staining necrotic mass was composed of remnants of the fetal placenta, fragments of the separated spongy layer, fibrin, erythrocytes, necrotic thrombosed blood vessels and nuclear debris (Figs. 8 and 9).

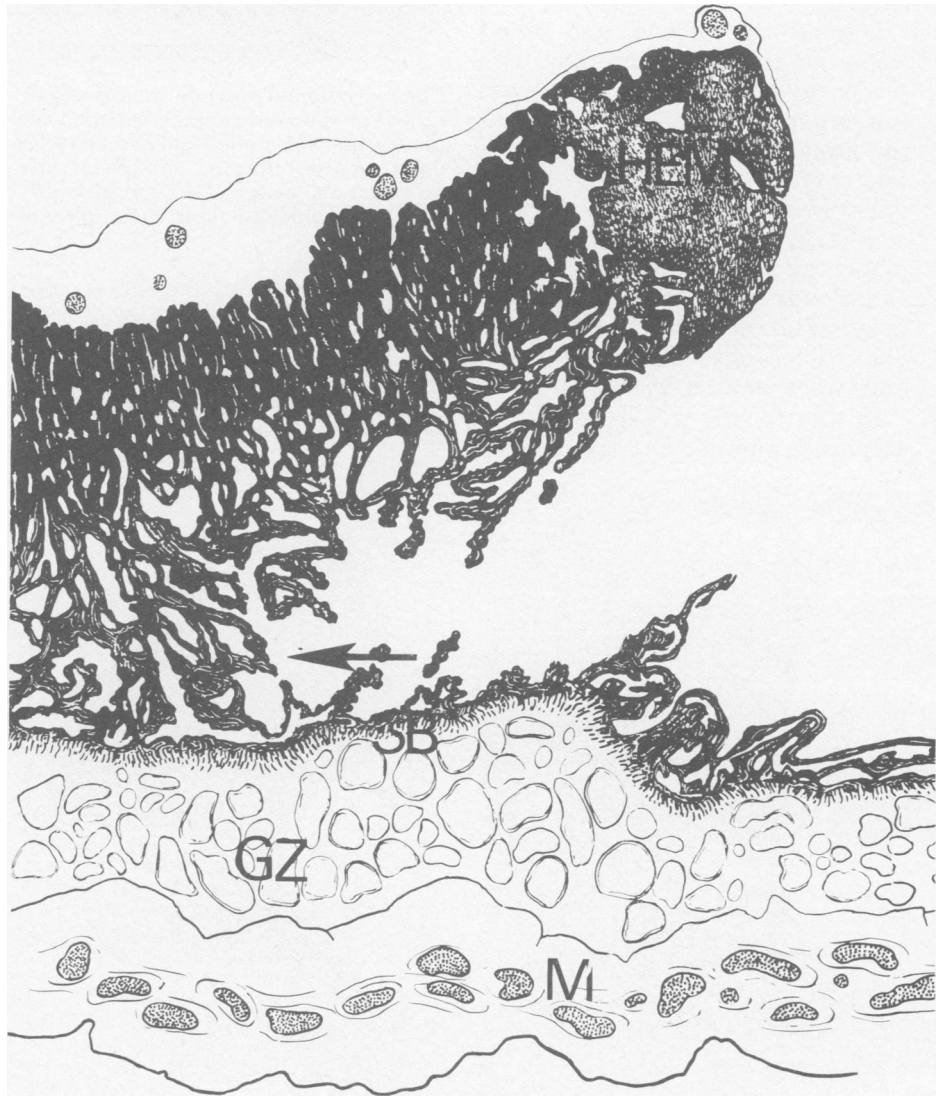

Fig. 7. A schematic drawing of partially separated placenta at parturition. The plane of separation of the fetal membrane lies in the spongy layer (arrow). The marginal hematoma (HEM) is at the upper right and the deep glandular zone (GZ) is just above the myometrium (M). The subplacental layer (SB) is between the deep glandular zone and the spongy layer.

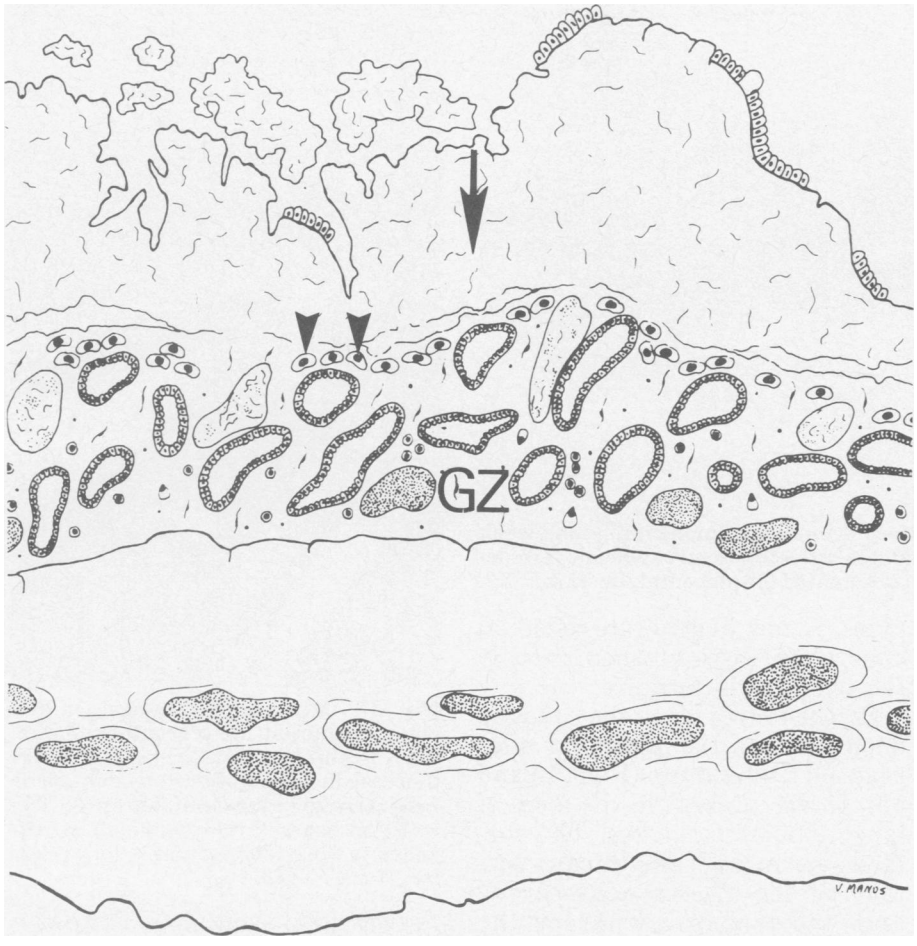

Fig. 8. A schematic drawing of uterus showing the microscopic changes in a uterus one week postpartum. The necrotic mass (arrow) is at the top and the deep glandular zone (GZ) below. Decidual cells (arrowheads) are between the necrotic mass and the deep glandular zone.

Large eosinophilic cells in moderate numbers were scattered in the upper loose connective tissue of the basal glandular zone of the lamina propria and under the necrotic mass. The cells were polygonal with an epithelioid appearance and abundant eosinophilic granular cytoplasm. The majority had one large, spherical nucleus although binucleate cells were not uncommon (Figs. 9 and 10). Many of these cells were degenerate with pyknotic or irregular nuclei and fully basophilic or vacuolated cytoplasm. These cells were considered to be either decidual cells (maternal) or trophoblastic cells (fetal), but due to their greater similarity to decidual cells have been designated as decidual cells.

During the second week collagen proliferation formed lobulated masses at the placental sites. The collagen appeared to be produced by proliferating fibroblasts in the lamina propria (Fig. 11). Small hemorrhages and scattered mononuclear cells were noted in the lamina propria. Decidual cells were seen in four out of six samples examined at this time and most of them were degenerate (Fig. 12).

During the third week there was an increase in the amount of colla-

Fig. 9. Microscopic changes in the endometrium at the placental site attachment during the first week postpartum. The necrotic mass on the surface (arrow) is composed of remnants of fetal placenta, fragments of the separated spongy layer, fibrin, erythrocytes and nuclear debris. Decidual cells (arrowheads) are in the upper endometrial lamina propria. H & E. X58.

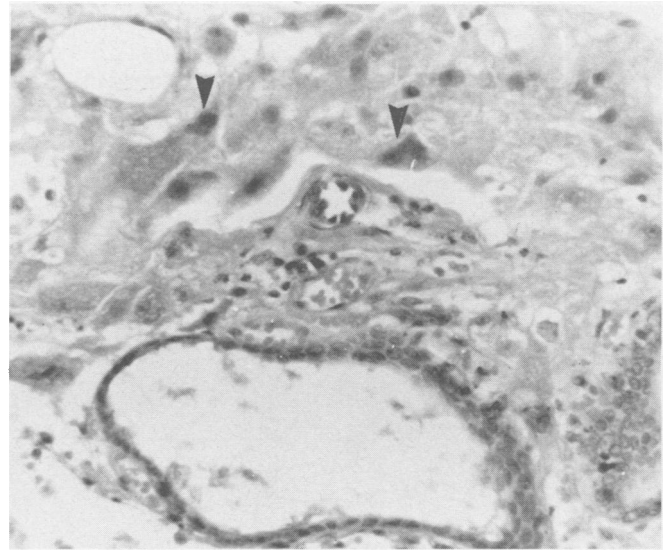

Fig. 10. Higher magnification of Fig. 9 to show the decidual cells (arrowheads). The decidual cells are large, polygonal, with an epitheliod appearance. Many of these cells are degenerated with a vacuolated cytoplasm and pyknotic nuclei. H & E. X232.

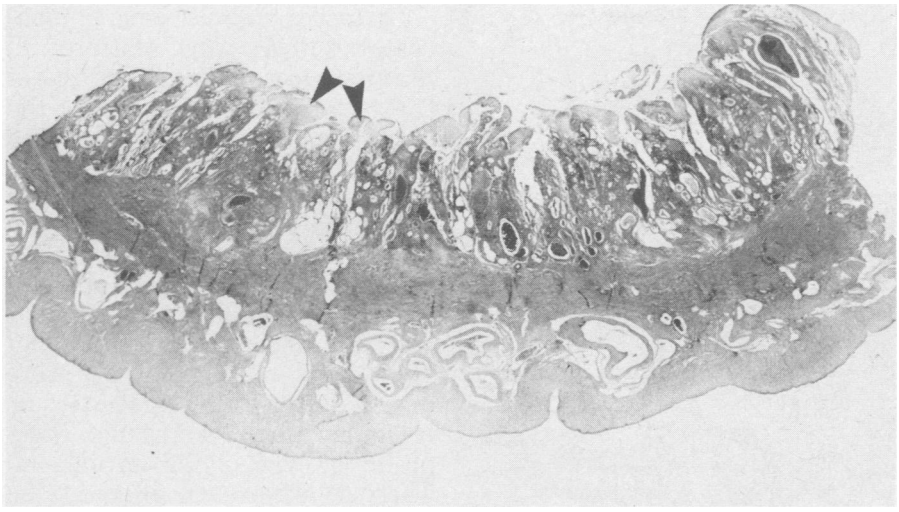

Fig. 11. Cross section of the uterine wall at the placental site during the second week postpartum. The myometrium is contracted. Collagen fibers (arrowheads) are deposited on the surface of the endometrial attachment of the placental site. H & E. X7.

gen covering the endometrium in the placental sites (Fig. 13). Mononuclear cell infiltration was greatly increased in the lamina propria. These cells were mainly macrophages, lymphocytes and a few plasma cells (Fig. 13). The endometrial glands were moderately dilated and their lumina contained cellular debris and erythrocytes. A few degenerate decidual cells were distinguished in only two out of eight samples.

By the fourth week the collagen deposition at the placental sites reached a maximum (Figs. 14 and 15). It was present in the form of large lobulated masses. The surface over most of the thicker portions of the masses was necrotic and hemorrhagic whereas the junction of these masses with the endometrium had more cellular and immature collagen. These collagen masses were covered by a single layer of foamy to eosinophilic staining columnar epithelial cells. This endometrial lining was interrupted over superficial parts of the masses where collagen was necrotic and sloughing. The endometrial glands were greatly dilated and their lumina were filled with mucus and sparse necrotic debris (Figs. 14 and 15). The mononuclear cell infiltration in the lamina propria was pronounced.

By the beginning of the fifth week the last and most important

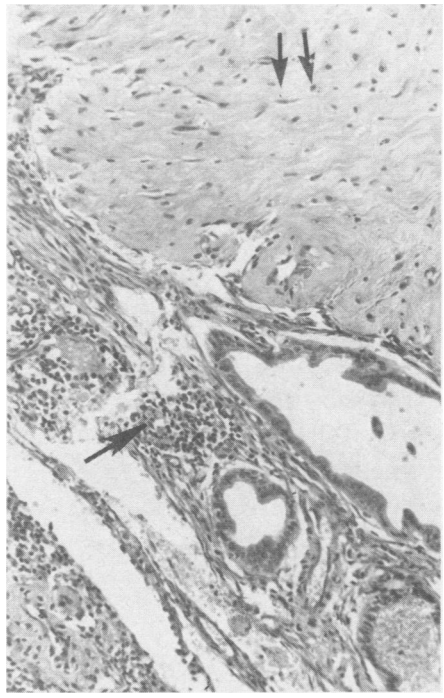

Fig. 13. Microscopic changes in the endometrium at the placental site during the third week. There is a great increase in the number of mononuclear cells (arrow) in the lamina propria. The collagen mass (arrows) which is attached to the endometrium is very cellular. H & E. X150.

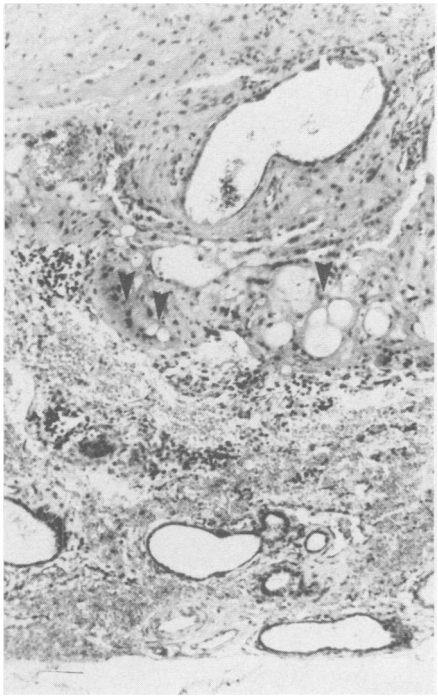

Fig. 12. Microscopic changes in the endometrium to show the decidual cells (arrowheads). Most of the decidual cells are degenerate and vacuolated. There are a few mononuclear cells in the lamina propria (left). H & E. X96.

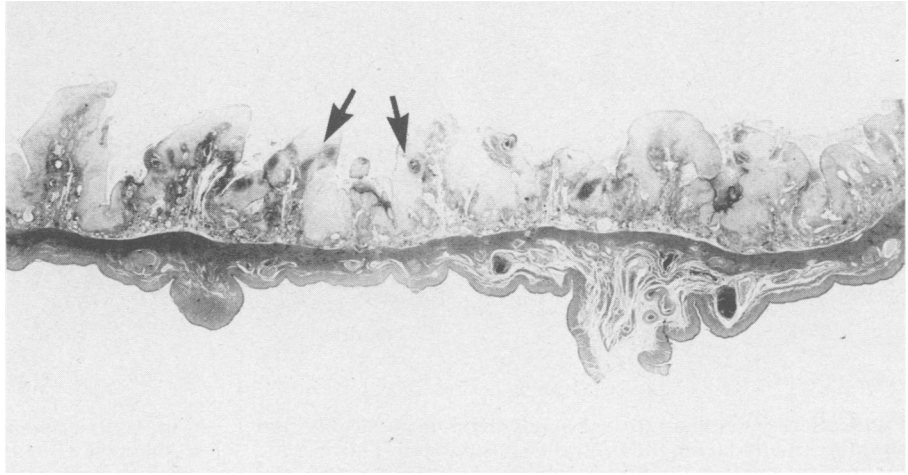

Fig. 14. Cross section of uterine wall at the site of placental attachment during the fourth week postpartum. Large amounts of collagen (arrows) have been deposited on the endometrial surface. The myometrium is thin and contracted. H & E. X5.

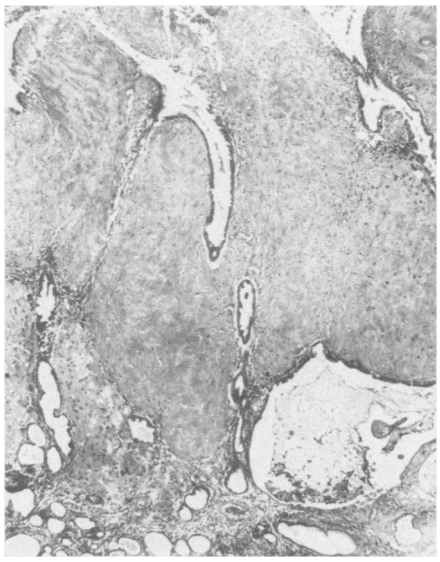

Fig. 15. Microscopic changes in the endometrium at the placental site during the fourth week. The surface of the collagen mass is necrotic and sloughing, whereas the more basal part is more cellular. The endometrial glands (right) are greatly dilated. H & E. X35.

stage of placental site involution started with massive denudation of the collagen masses into the lumen. Sloughing was at the level of attachment to the endometrial lamina propria (Fig. 16). The area of detachment was soon regenerated and covered by a single layer of columnar epithelial cells. Most of the uterine glands were small, widely separated and a few were moderately dilated. A great increase in nuclear density of fibroblasts, macrophages, lymphocytes, and few plasma cells occurred around the uterine glands and beneath the endometrial epithelium.

Changes similar to those observed during the fifth week were seen during the sixth and seventh week postpartum, but were more prominent. Most of the collagen masses were detached from the endometrium and found in the uterine lumen (Figs. 17 and 18). The majority of the uterine glands were normal in size and shape (Figs. 17 and 18) and had narrow lumens and darkly stained cells with basal nuclei. Mononuclear cell infiltration was greater than during the previous period.

By the commencement of the eighth week almost all of the colla-

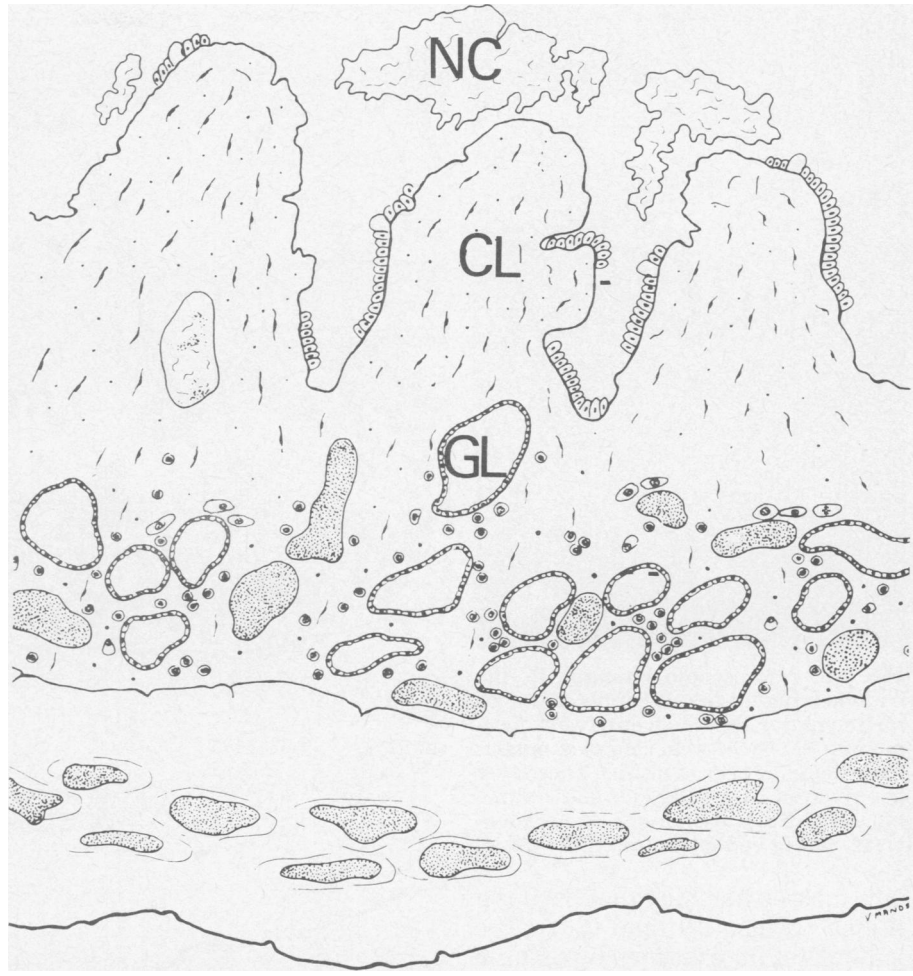

Fig. 16. Diagrammatic representation of findings in Fig. 14 and 15. Necrotic masses (NC), collagen masses (CL), dilated endometrial glands (GL).

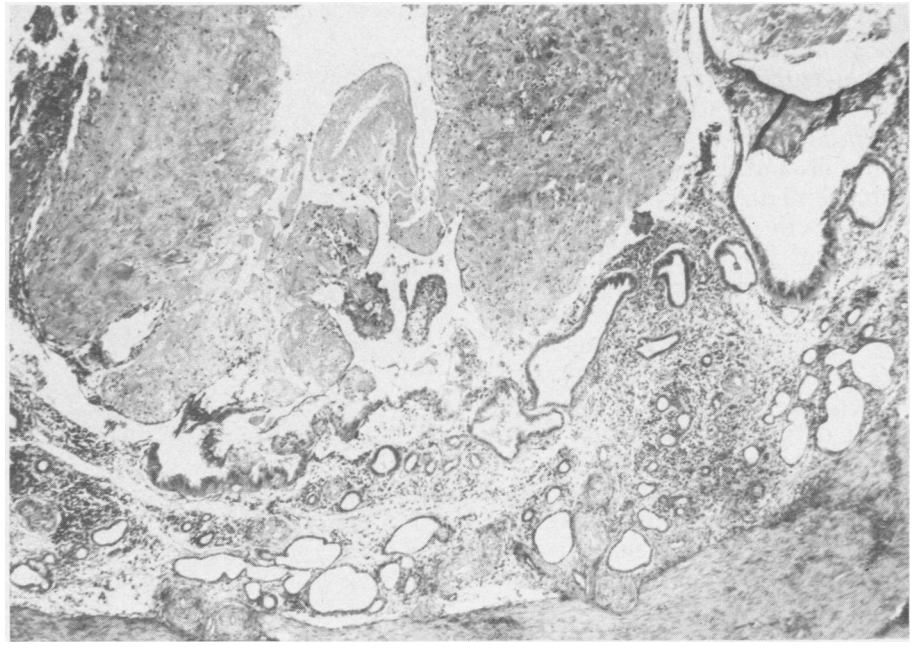

Fig. 17. Microscopic changes at the site of placental attachment during the fifth week postpartum. Massive sloughing of the collagen masses is underway. H & E. X51.

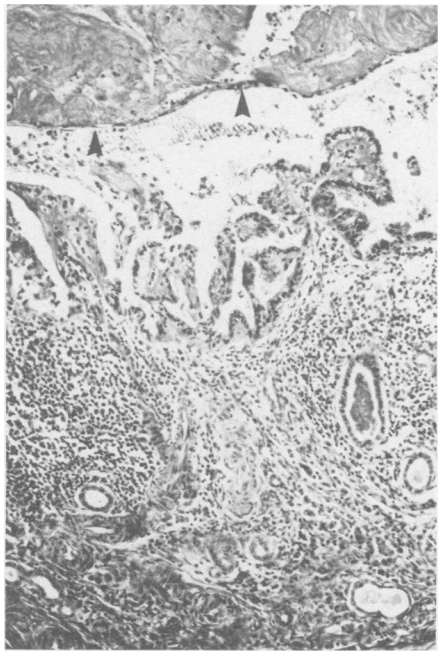

Fig. 18. Microscopic changes in the endometrium at the site of placental attachment during the seventh week postpartum. Most of the collagen masses have sloughed (arrowheads). These sites are covered by small columnar epithelial cells. Note abundance of mononuclear cells in endometrium. H & E. X86.

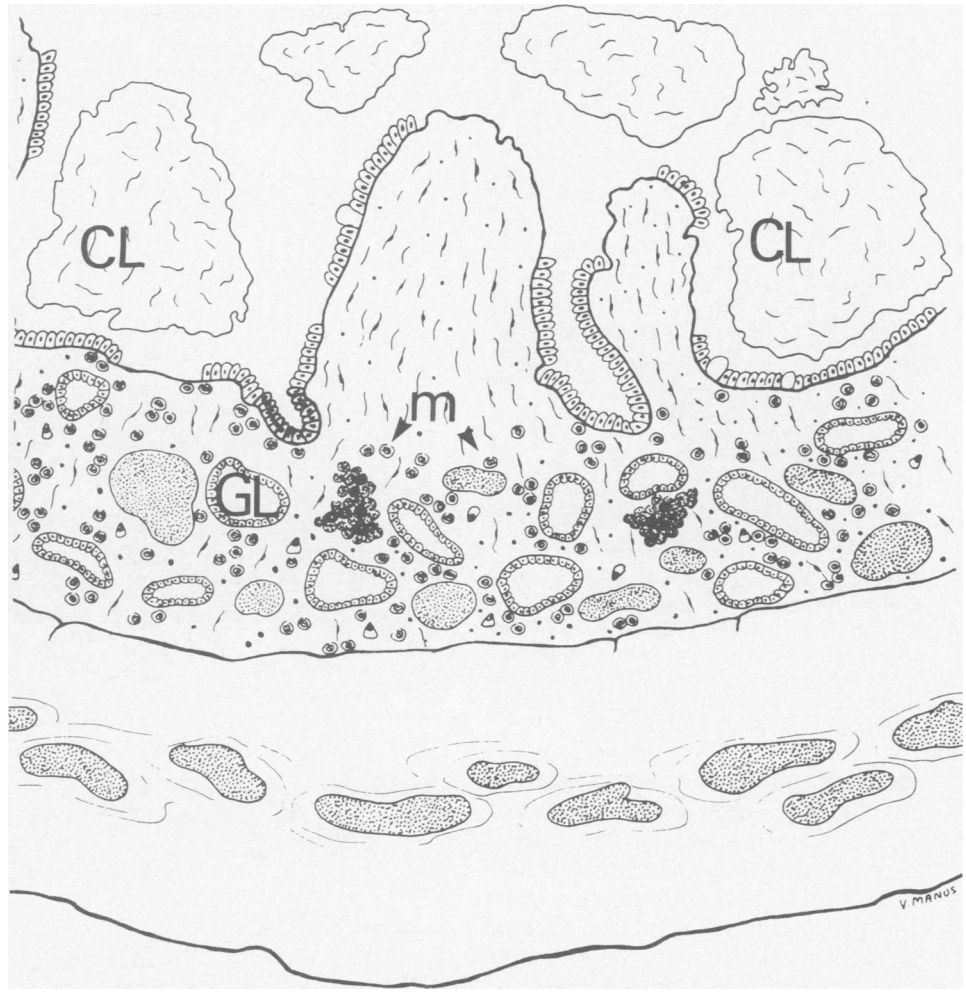

Fig. 19. Summary of the changes during the fifth, sixth, and seventh weeks. Collagen masses (CL) sloughed into the lumen. Endometrial glands (GL) in normal size and shape. Mononuclear cells (M) are present in large numbers.

gen masses had sloughed into the lumen (Figs. 19 and 20). The endometrium was lined by a single layer of foamy columnar epithelial cells. These cells were continuous with smaller cells with basophilic cytoplasm and darkly stained basal nuclei at the mouths of the regenerated uterine glands (Figs. 21 and 22).

By the ninth week sloughing was complete but the regeneration and replacement of the endometrial epithelium continued from the mouth of the uterine glands and from the regenerated epithelial cells on the surface (Fig. 23). This process continued until the end of the twelfth week when the whole endometrium was lined by a single layer of small cuboidal or columnar cells with basophilic staining cytoplasm and darkly stained basal nuclei. The uterus was classified as anestrus from the thirteenth week postpartum. The brown color of the involuted placental sites was mainly due to large numbers of hemosiderin-laden macrophages.

A summary of the histological

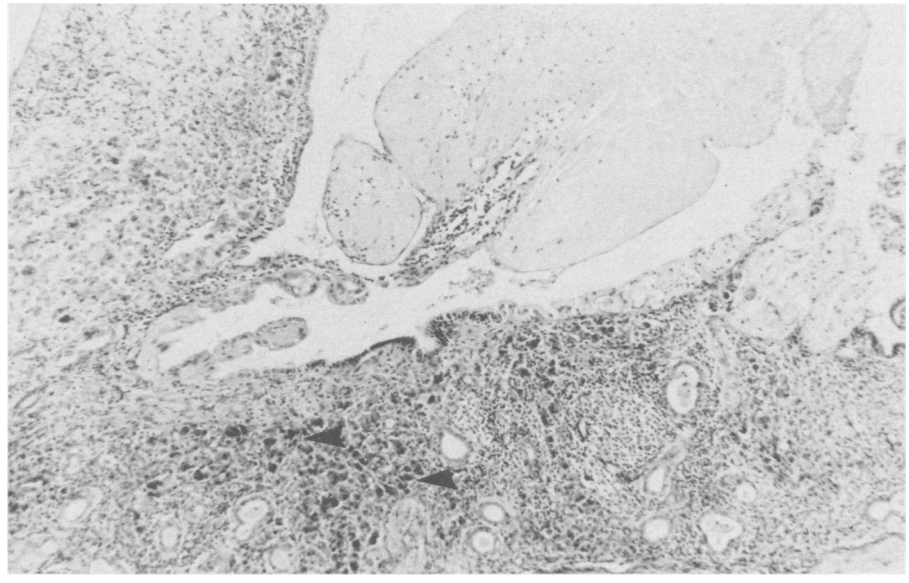

Fig. 20. Microscopic changes in the endometrium at the placental site during the eighth week. All of the collagen masses have sloughed into the lumen. The endometrium is lined, in some parts, by large foamy columnar epithelial cells. Hemosiderin-laden macrophages in the lamina propria are increased in number. H & E. X75.

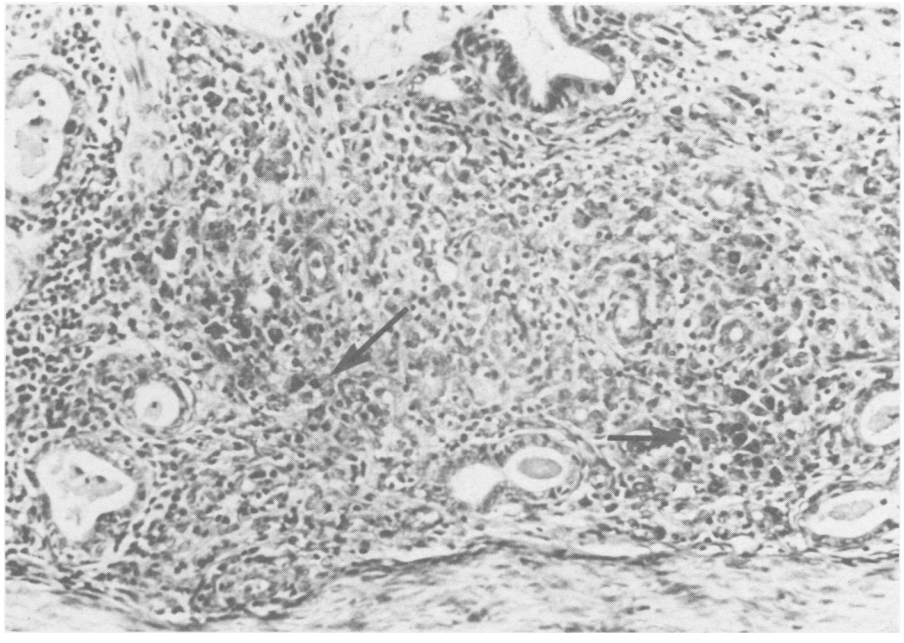

Fig. 21. Higher magnification of Fig. 20 to show the replacement of the tall foamy columnar epithelial cells by small epithelial cells with basophilic staining cytoplasm, emanating from the mouths of the glands. Note hemosiderin-laden macrophages (arrows). H & E. X240.

changes is given in Table II. The application of the trichrome method of staining was very useful to determine the amount of collagen deposited on the placental sites at different time periods postpartum. Semiserial sections examined from selected cases revealed that the glands opened to the lumen without any narrowing or blockage of the ducts and no substantial microscopic differences were seen in different areas of the same placental site.

Interplacental Site Endometrium — During the first week postpartum the interplacental endometrium was composed of primary and secondary folds. The primary folds were formed by the greatly edematous lamina propria which contained a few lymphocytes and plasma cells (Figs. 24 and 25). The secondary epithelial folds were wrinkled and consisted of long columnar epithelial cells (Fig. 26). The epithelial cells varied in size and had a foamy cytoplasm in which an abundance of lipid droplets were seen. Signs of degeneration were present in some cells

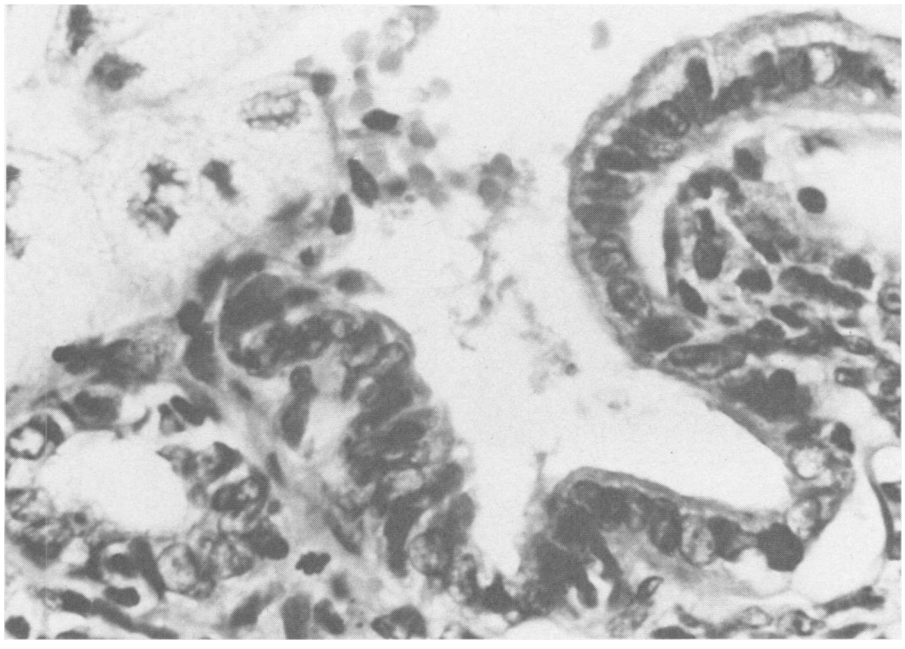

Fig. 22. Higher magnification of Fig. 21. Small cells with basophilic cytoplasm and darkly stained nuclei from the mouth of the gland are replacing the tall foamy columnar epithelial cells lining the endometrium. H & E. X630.

TABLE II. Microscopic Changes in the Placental Sites at Different Postparturient Time Periods in the Dog

Week	Appearance
1	Eosinophilic necrotic layer covered the placental sites Few decidual like cells between the necrotic layer and basal endometrial glands
2	Collagen fiber proliferation from the endometrial lamina propria Slight mononuclear cell infilitration in the lamina propria A few degenerate decidual cells
3 and 4	Increased amount of collagen lining the endometrium as irregular lobulated masses Increased mononuclear cell infiltration Dilated endometrial glands Absence of decidual cells
5	Sloughing of collagen masses Great increase in mononuclear cell infiltration Glands regenerating and returning to normal size
6 and 7	Continued sloughing of collagen masses Extensive mononuclear cell infiltration Endometrial glands normal in size
8	Sloughing of collagen masses completed Replacement of endometrial lining from the mouth of the regenerated glands Endometrial epithelial cells small with basophilic staining cytoplasm
9-11	Continued replacement of the endometrial lining
12	Endometrium completely lined by a single layer of small cells with basophilic staining cytoplasm
13	Uterus at anestrus stage

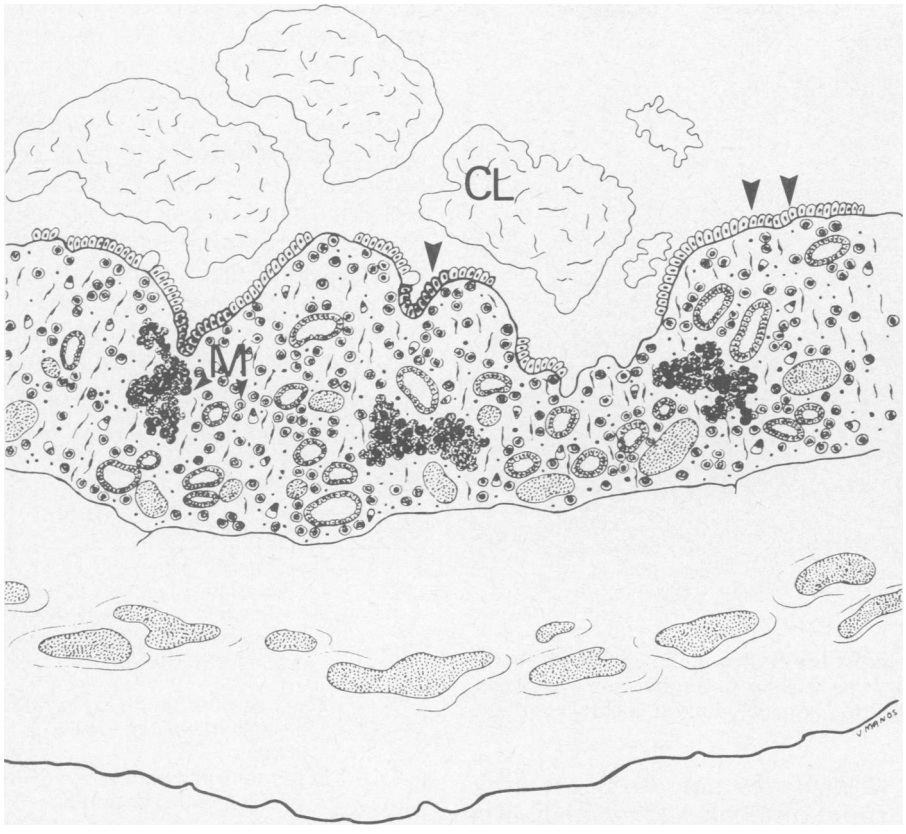

Fig. 23. Summary of changes during the ninth week. Complete sloughing of the collagen masses (CL). Marked increase in the number of mononuclear cells (M) and hemosiderin-laden macrophages. Endometrium covered by a single layer of epithelial cells (arrow).

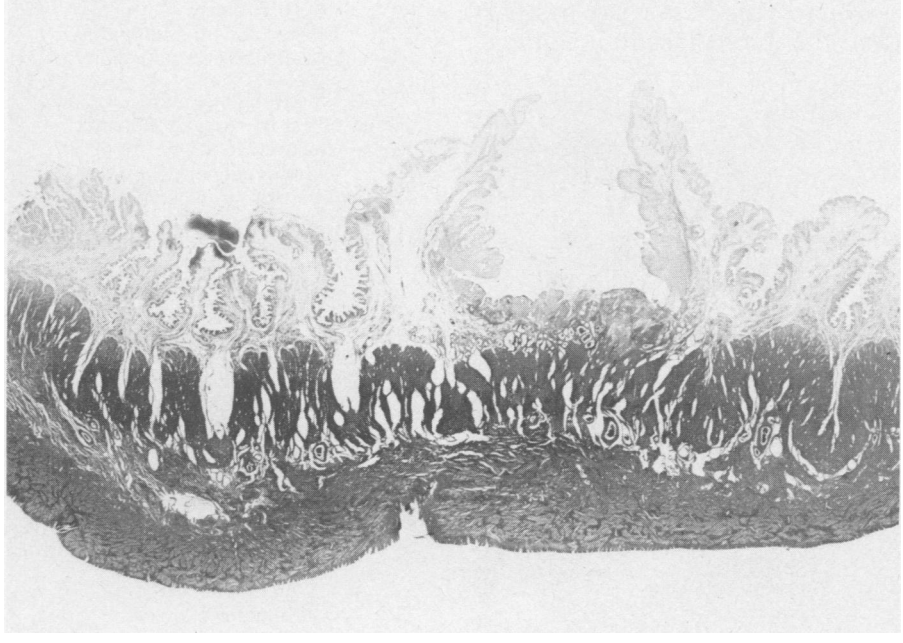

Fig. 24. Longitudinal section through the uterine wall during the first week postpartum, showing both a site of placental attachment (just to the right of the center) and interplacental areas of the endometrium. The placental site is covered with an eosinophilic necrotic mass; the edges are greatly folded and the primary folds which are formed from the lamina propria are visible. The myometrium is thick and contracted. H & E. X4.

Fig. 25. Microscopic changes in the endometrium at the interplacental sites during the first week postpartum. The endometrium is composed of primary and secondary folds. The primary folds are formed by the edematous lamina propria. The secondary folds are formed by wrinkled epithelial lining. H & E. X55.

while others were greatly distended with mucus and had pyknotic nuclei. The endometrial lumen was still intact and no epithelial desquamation was seen.

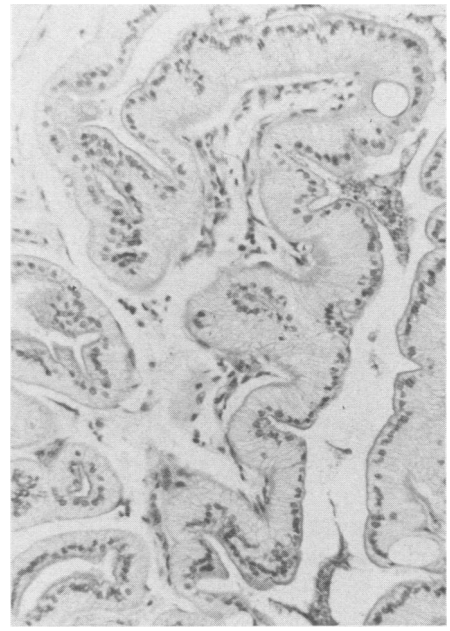

Fig. 26. Higher magnification of Fig. 25. The epithelial cells are tall, columnar and some of them are degenerate. Note apical placement of nuclei. H & E. X150.

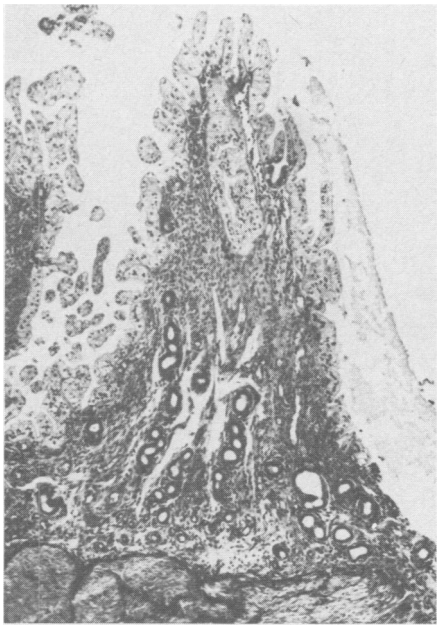

Fig. 27. Microscopic changes in the endometrium at the interplacental sites during the sixth week. Sloughing of the endometrial epithelial fold has continued. The lamina propria is less edematous and more cellular. H & E. X59.

By the second and third week the lamina propria was less edematous. By the fourth week massive sloughing of the epithelial cells occurred into the lumen. The lamina propria was less edematous and more cellular with an increase of the mononuclear cell infiltration. A few of the endometrial glands were slightly dilated, whereas the remainder were small, involuted and lined by low columnar or cuboidal epithelial cells.

During the fifth and sixth week sloughing of these secondary epithelial folds continued and remnants were seen in the lumen. Only small folds of foamy epithelial cells were seen covering the endometrium (Fig. 27). The endometrial lining near the mouth of the glands was replaced from the mouth of the involuted endometrial glands (Fig. 27). The lamina propria was highly cellular due to increased numbers of fibroblasts, macrophages, plasma cells, and a few neutrophils (Fig. 27).

During the seventh and eighth week exfoliation of the degenerate foamy epithelial cells into the lumen continued. The endometrium was covered by a single layer of large foamy and degenerating epithelial cells. Many nuclei were irregular and pyknotic (Fig. 28). Replacement of the exfoliated parts from the mouths of the glands continued. By the ninth week many areas of the endometrial lining were replaced by a single layer of small cells with basophilic staining cytoplasm. This process continued throughout the tenth and eleventh week postpartum and by the end of the twelfth week the whole endometrium was lined by a single layer of small cuboidal or columnar cells with basophilic staining cytoplasm.

Myometrium — During the first week postpartum the myometrium was thick and contraction of the outer longitudinal muscular layer produced folds on the serosal surface. The stratum vasculare was very thick due to the large amount of collagen fibers and greatly dilated and thickened blood vessels. Interlacing bundles of collagen fibers appeared between the muscle bundles of both muscular layers (Figs. 29 and 30).

By the fourth week postpartum the myometrium was thin, and the muscle fibers were smaller. The collagen in the stratum vasculare was greatly reduced in amount and only a few fibers were seen

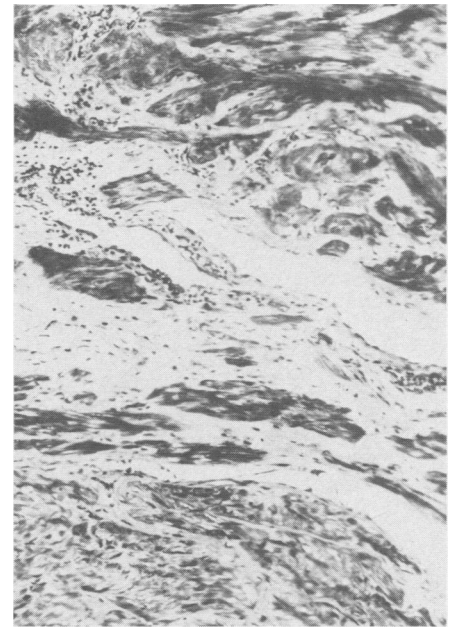

Fig. 29. Myometrium during the first week postpartum. The myometrium is thick and a large amount of collagen is in the stratum vasculare. Interlacing bundles of collagen fibers (light fibers) occur between the muscle fibers (dark fibers) in the inner circular and outer longitudinal layers. The blood vessels in the stratum vasculare are dilated. Trichrome X150.

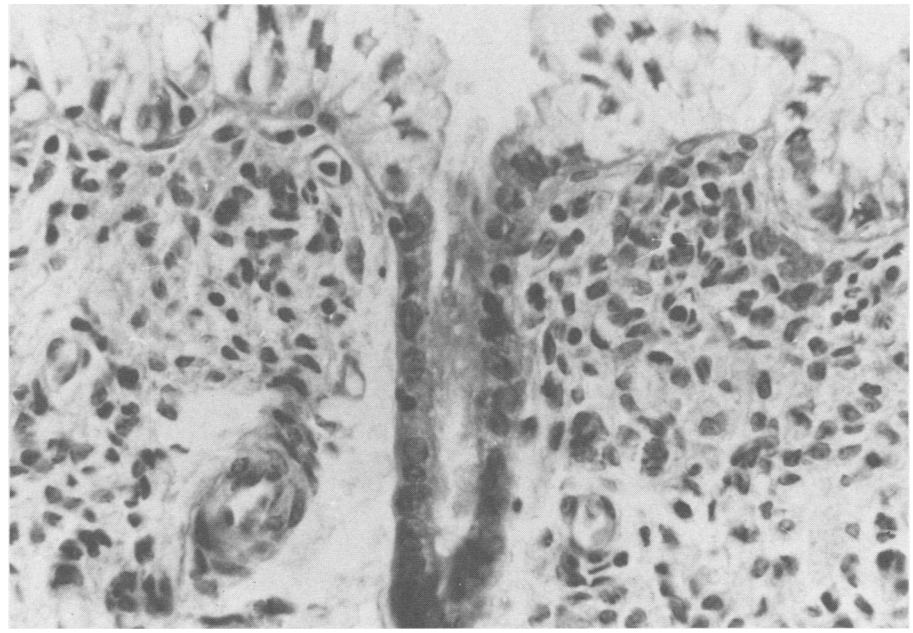

Fig. 28. Microscopic changes in the endometrium at the interplacental site, during the eighth week. The endometrium is lined by a single layer of large foamy and degenerating epithelial cells, many of which have irregular and pyknotic nuclei. Replacement of the exfoliated epithelium occurs from the mouth of the glands. H & E. X475.

Fig. 30. Portion of the longitudinal muscle layer during the first week postpartum showing the large amount of collagen fibers (light fibers) interlaced between the muscle fibers (dark fibers). Masson trichrome X150.

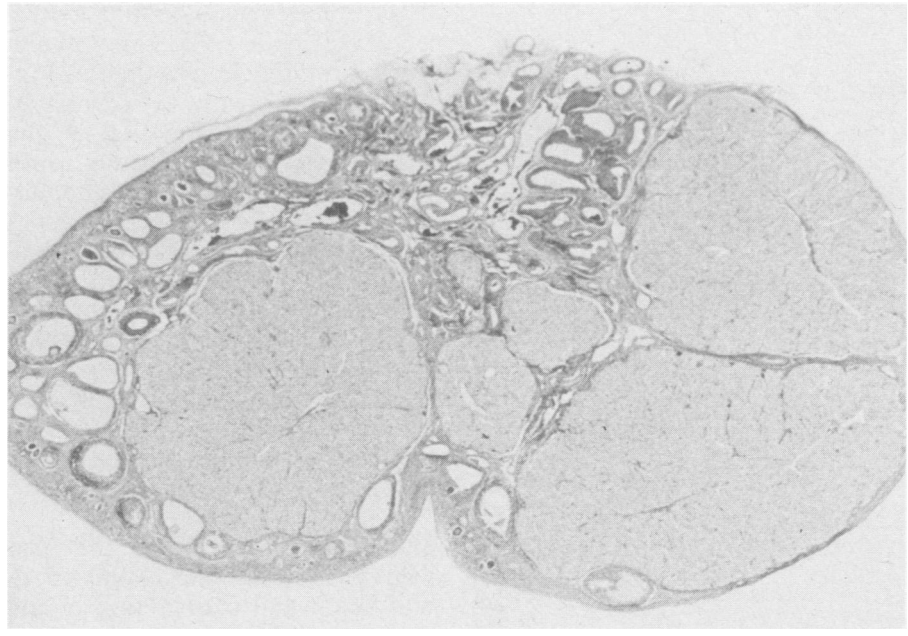

Fig. 32. Retrogressing corpus luteum during the first week postpartum. The corpora lutea are large and easily recognizable. The radiating clear spaces are less evident. Masson trichrome X10.

between the muscle bundles. The arteries in the stratum vasculare were greatly contracted. The intima and media layers were thickened, but the collagen in the adventitia was decreased in the amount. By the sixth week the myometrium appeared more cellular due to the decrease in the volume of the cells (Fig. 31).

Luteal Regression — During the first week postpartum, the corpora lutea were still large (Fig. 32). However, signs of cellular degeneration were evident within the corpus luteum. Many of the luteal cells contained numerous vacuoles and eccentric pyknotic nuclei (Fig. 33). The radiating spaces in the center were less evident (Fig. 32) in comparison to the corpus luteum of pregnancy. By the fourth week the corpora lutea were in the medulla, smaller and degenerative changes were more evident. Most luteal cells contained variable sized vacuoles and the nuclei were irregular in shape and many were pyknotic (Figs. 34 and 35). A few collagen fibers were noticed between the luteal cells. The blood vessels at the periphery were greatly thickened and many of the capillaries within the corpus luteum had disappeared. The most salient change during the sixth week was a great increase in collagen fibers in the corpus luteum. Very few capillaries within the corpus luteum were seen. By 12 weeks, corpora lutea were very small and located deeply in the medulla (Fig. 36). Most luteal cells were filled with one clear vacuole and their nuclei were degenerate (Figs. 37 and 38). Collagen fibers between the luteal cells were increased. By 20 weeks, the corpus luteum was greatly shrunken and contained large amounts of collagen fibers and fibroblasts. Luteal cells appeared as irregular masses of vacuolated pigmented cells.

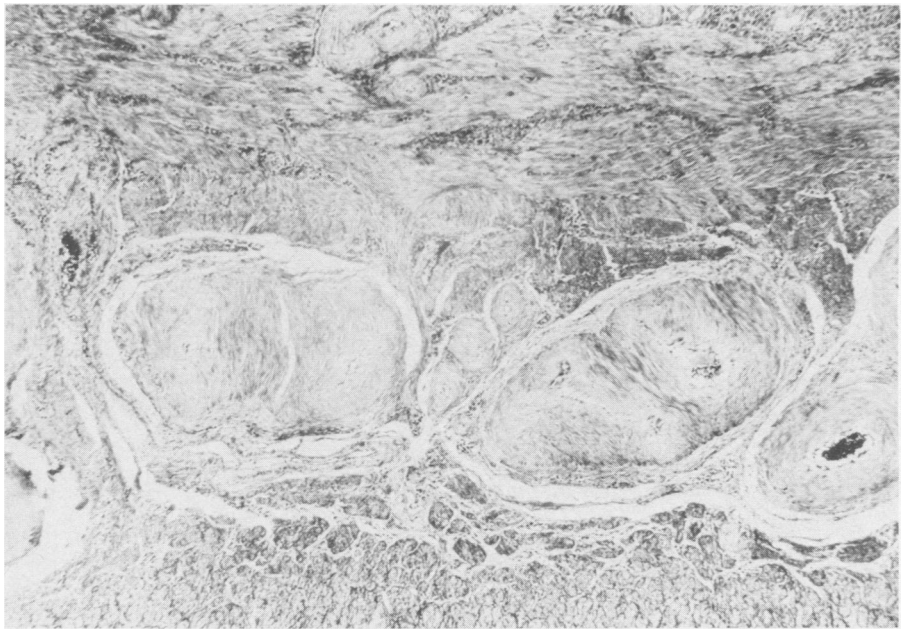

Fig. 31. Myometrium during the sixth week postpartum. The myometrium is thin, contracted and more cellular. The blood vessels are thickened and contracted (arrows). Masson trichrome X75.

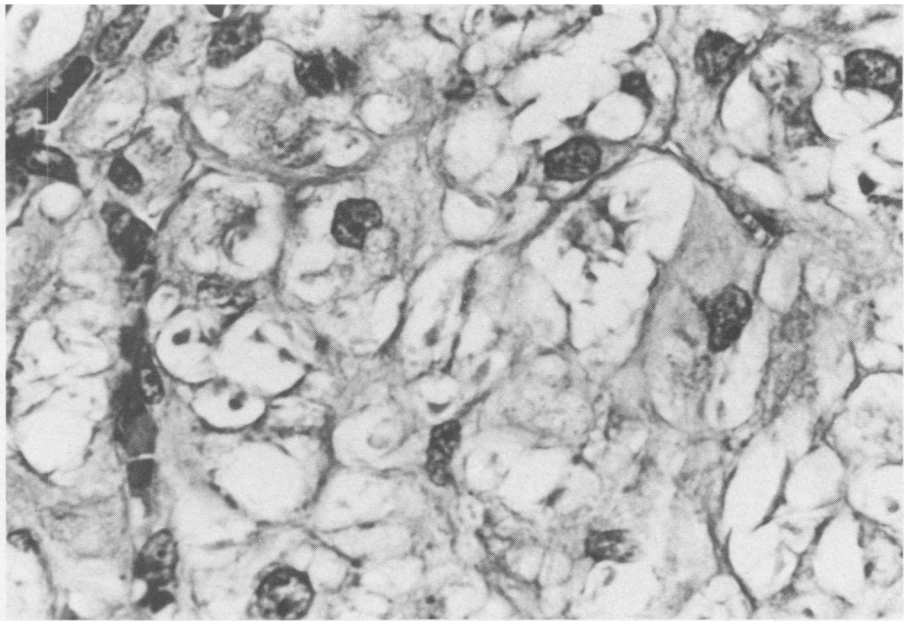

Fig. 33. Higher magnification of Fig. 32. Many healthy appearing luteal cells are seen. Most of the luteal cells have cytoplasmic vesicles and a few nuclei are pyknotic. Masson trichrome X475.

Progesterone Assay — Plasma progesterone levels were obtained from bitches at different time periods postpartum (Table III). The progesterone level was very low (below 2 ng/mL except in three cases . . .) during the main reparative process of the endometrium (up to eight weeks postpartum). The progesterone level was very high (9.2 ng/mL) in one case (four days postpartum) in comparison to other cases in the same period. At 40 days postpartum the same bitch had 2.4 ng/mL plasma progesterone level and was diagnosed as subinvolution of placental sites grossly and histologically (1).

Fig. 34. Retrogressing corpus luteum during the fourth, fifth and sixth week postpartum. The corpora lutea are small in size and located deep in the medulla. Masson trichrome X8.

DISCUSSION

There are several new findings in this paper: the plane of separation of the placenta at parturition, the collagen fiber proliferation at the placental sites during the first four weeks postpartum and then their sloughing, the differences in the involution between the placental site and interplacental site endometrium, and the myometrial involution in the postpartum dog.

The objective of this investigation was to determine the gross and microscopic changes in the normal postpartum uterus in the dog. Postpartum involution is a slow process and 12 weeks are necessary for completion. The major endometrial changes are seen during the first nine weeks postpartum, during which a massive breakdown of cellular material and connective tissue occurs. The secondary endometrial changes starting at the ninth week and ending by the twelfth week, are of minor importance. These were characterized by a change in shape and size of the epithelial cells lining the endometrium.

The present study indicates that during the first week postpartum the placental sites can be identified easily by gross examination of the mucosa. This is in contrast to the report (3) which indicated difficulty in gross recognition of the placental sites during the first week postpartum, due to marked contraction of the myometrium.

Anderson and Simpson (3) and Jochle and Andersen (13), indicated that exfoliation of the endometrium stopped between eight to 10 weeks postpartum and was followed by repair. In contrast the present study indicates that both exfoliation (or sloughing) and repair of the endometrium occur together and by the ninth week most of the endometrium at the placental sites and interplacental areas was repaired.

Most of the large cells with abundant cytoplasm which were seen during the first and second week postpartum were considered to be decidual cells. These cells were located in the upper loose

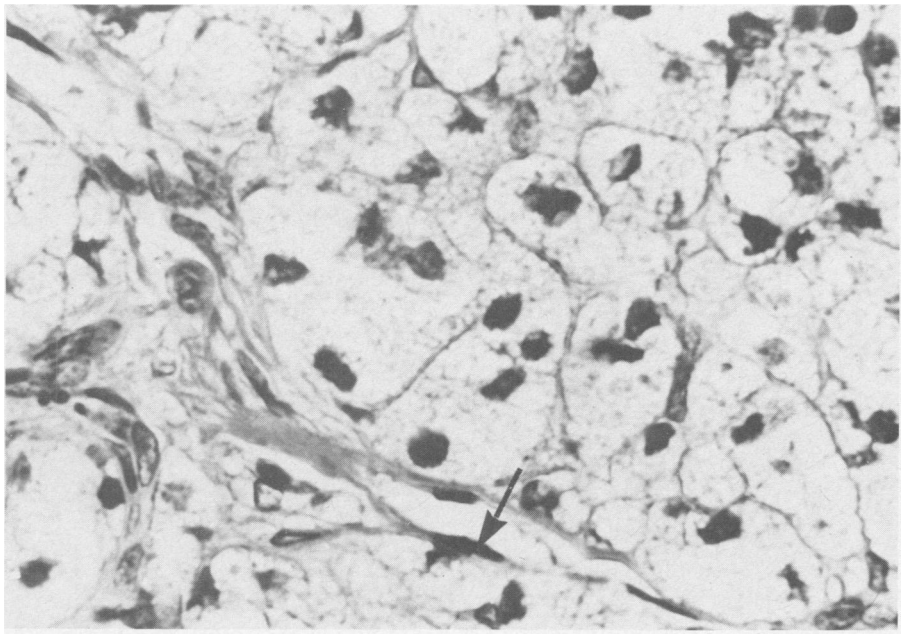

Fig. 35. Higher magnification of Fig. 34. The luteal cells are vesicular with distorted pyknotic nuclei. Many fibroblasts lie between the luteal cells. Arrow indicates endothelial cell. Masson trichrome X630.

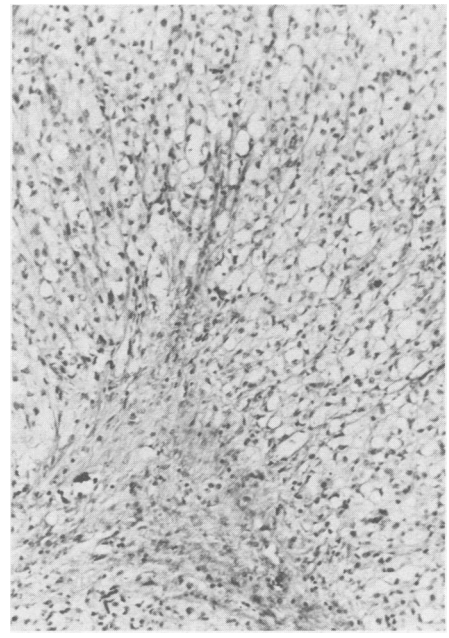

Fig. 37. Higher magnification of Fig. 36 near the connective tissue core. Most of the luteal cells are filled with one clear vacuole and the fibrous tissue has spread deeply between the luteal cells. Masson trichrome X96.

connective tissue of the basal glandular zone. They tended to resemble trophoblastic cells, especially the more degenerate cells. However, they were considered to be decidual cells. This decision was based on the fact that these cells were located at the same level as the subplacental layer during pregnancy which has been demonstrated to be composed of compact decidual cells (2). In addition, the separation of the placenta at parturition has been shown to be just above the subplacental layer, and remnants of decidual cells in the endometrium from the subplacental layer have been seen shortly after parturition (1). Also, separation of the placenta has been shown to occur at the base of the spongy zone (maternal part) and not at the junctional zone where the trophoblast cells are usually seen during pregnancy (1). The presence of trophoblastic cells has been observed in the endometrium and myometrium at the placental site

Fig. 36. Retrogressed corpus luteum 90 days postpartum. The corpora lutea are small, shrunken and lie deep in the medulla. Masson trichrome X6.

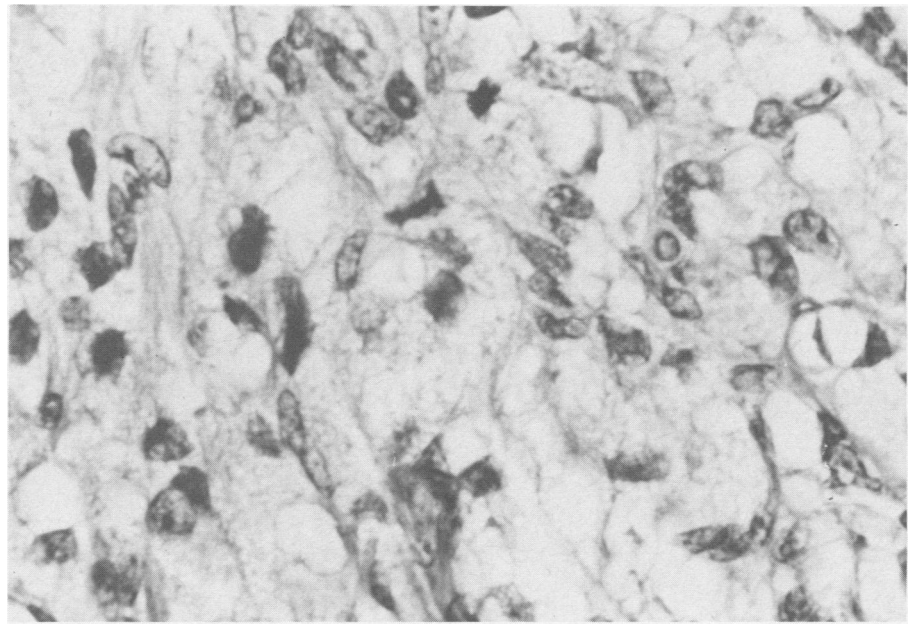

Fig. 38. Higher magnification of Fig. 37. Large numbers of fibroblasts replacing the degenerate luteal cells. H & E. X630.

in one case four days postpartum (1).

Collagen proliferation on the endometrial surface at the placental sites is part of the repair process of the injured and denuded epithelial surface. Many factors could be responsible for the decrease of collagen after the fourth week postpartum. Continuous exfoliation of the degenerate collagen into the lumen has been shown to occur with cessation of fibroblast proliferation when the healing process is finished. Postpartum uterine contraction is considered to be important in the complete sloughing of collagen masses into the lumen. The blood vessel contraction after parturition may have caused a decrease in the blood supply to the collagen masses. This may lead to ischemic degeneration and exfoliation of the masses. Also part of the collagen may be resorbed by macrophages. Mononuclear cells increase in number dramatically after the fourth week postpartum, i.e. the same time as the collagen is decreasing. In the rat endometrial phagocytes show intense postpartum phagocytic and pinocytotic activity accompanied by the development of abundant lysosomes (6). The material contained in the phagosomes represented byproducts of collagen catabolism (12).

In contrast, an extensive and rapid breakdown of collagen was noticed in the myometrium shortly after parturition. This was considered to be a normal event during postpartum involution of the mammalian uterus; rapid and massive breakdown of tissue occurs. Involution involves resorption of tissue components such as muscle and connective tissue (4). During the last days of pregnancy, there was a gradual buildup of acid hydrolases, followed by further increases particularly during the postpartum period (20). The collagenolytic activity of the postpartum uterus is greatly affected by the level of steroid hormones in rats. Injection of large doses of estrogen or progesterone into the postpartum rat retarded collagenolytic activity and uterine involution (9, 11). In contrast ovariectomy at the time of parturition accelerated involution and the loss of collagen (17). It appears that the decrease in progesterone in the rat may act as a signal for the involution of the uterus and the accompanying increase of collagenase activity (11).

The pregnancy in the dog is considered to be corpus luteum-dependent and the ovary is the main source of progesterone throughout the pregnancy (8). The luteal phase in the dog lasts about two months even in the absence of pregnancy, and the uterus is, therefore, under the influence of progesterone. Plasma progesterone rises rapidly after the onset of estrus and attains maximum levels at 25 to 30 days after the commencement of the estrus without regard to pregnancy (7, 10, 13).

Our results indicated that progesterone level is very low in the postpartum bitch. The postpartum collagen breakdown in the myometrium and interplacental site endometrium may be related to the progesterone withdrawal after parturition. On the other hand the injury to the placental site at parturition may induce the process of tissue repair which is characterized by the collagen proliferation on the placental site surface. In this case collagen proliferation would be an independent process, not affected by the postpartum hormonal withdrawal.

TABLE III. Plasma Progesterone Levels at Different Postparturient Time Periods in the Dog

Case #	Days (or wk) Postpartum	Progesterone Level ng/mL	Method of Isolation
1655-77	2 (or 1st wk)	0.8	CPB[a]
943-77	4 (or 1st wk)	3.0	"
1602-77	4 (or 1st wk)	9.2	"
4-78	4 (or 1st wk)	2.0	"
189-77	11 (or 2nd wk)	1.1	RIA[b]
148-77	10 (or 2nd wk)	0.2	"
237-77	21 (or 3rd wk)	0.3	RIA
500-77	15 (or 3rd wk)	0.2	"
228-77	22 (or 4th wk)	0.3	RIA
536-77	25 (or 4th wk)	0.5	"
544-77	22 (or 4th wk)	0.8	"
530-77	26 (or 4th wk)	0.3	"
543-77	25 (or 4th wk)	0.6	"
594-77	30 (or 5th wk)	0.9	RIA
529-77	30 (or 5th wk)	0.6	"
17-78	40 (or 6th wk)	2.4	CPB
150-77	49 (or 7th wk)	1.3	RIA
930-77	44 (or 7th wk)	0.5	CPB
1059-77	46 (or 7th wk)	0.5	"
1062-77	48 (or 7th wk)	1.2	"
1071-77	53 (or 8th wk)	1.2	CPB
1072-77	54 (or 8th wk)	0.5	"

[a]Competitive protein binding assay method
[b]Radioimmunoassay method

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